

A COMPARISON OF THE SPIROCHETE OF YELLOW FEVER
(*LEPTOSPIRA ICTEROIDES* NOGUCHI) WITH
THE *LEPTOSPIRA* OF WEIL'S DISEASE.

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In the year 1923, yellow fever prevailed in Bucaramanga, the capital of the Department of South Santander in Colombia, South America. The diagnosis, which was made at the time by a group of local physicians,¹ was confirmed by a commission of the International Health Board of the Rockefeller Foundation.² This commission conducted its work in our laboratory at Chapinero (Bogota). The confirmation was made serologically by Pfeiffer tests in guinea pigs furnished by us, cultures of *Leptospira icteroides* having been forwarded by Dr. Noguchi. It then became an aim of the laboratory to be prepared to determine a diagnosis of yellow fever by Pfeiffer tests. For the purpose we made an attempt to grow our own cultures of *Leptospira icteroides* from the only guinea pig that survived the Pfeiffer tests of the commission.

The animal (Guinea Pig 1) had been inoculated intraperitoneally by Dr. Pothier on May 16, 1923, with a rich culture of *Leptospira icteroides* (grown on Noguchi medium) together with human serum from a suspected case of yellow fever. On May 24, 1923, 8 days later, heart's blood was taken from the inoculated animal and placed in Ungermann medium, containing rabbit serum inactivated for 30 minutes at a temperature of 58–60°C. and covered with a protective layer of paraffin. On June 9, 1923, the culture showed an abundant growth of lepto-

¹ Drs. Daniel Peralta, Martin Carvajal, Francisco Pradilla Gonzalez, Andres Gomez, Julio Valdivieso, Roberto Serpa, and Louis Ardila Gomez, who in a publication issued in April, 1923, substantiated their findings by clinical histories.

² Drs. J. H. White, Pothier, and Wenceslao Pareja, who were sent in response to a request made by the Government of Colombia, through the Director of Hygiene, Dr. Pablo Garcia Medina.

spira, and we were, therefore, in a position to undertake serological tests for yellow fever.

In our subsequent work we employed the pure or a modified form of the Ungermann culture medium and adopted the following procedure. During the early months, we made weekly transfers to guinea pigs. Certain animals were inoculated with *Leptospira icteroides* (Noguchi), and others, for purposes of comparison, with *Leptospira icterohæmorrhagiæ* (Weil's disease). Series of cultures were made in both instances, at first with heart's blood, or suspensions of liver and kidney. Later on, cultures were inoculated only with heart's blood. In every case, the pathogenic material was taken on the day of maximal fever and cultured promptly.

With the cultures thus obtained, guinea pigs were again inoculated intraperitoneally, and from the heart's blood of the infected animals, other cultures were made for the inoculation of fresh guinea pigs, and so on. It was observed that the virulence of the spirochetes decreased gradually in the process of passage, so that finally, in August, 1923, after about 3 months of transfer, no animals died of the infection, although large numbers of spirochetes were injected. During the subsequent months, an animal would now and then exhibit a slight degree of the characteristic icterus, but notwithstanding the high fever (up to 41°C.), all survived the infection. Ultimately no jaundice was perceptible, but cultures could still be obtained from the heart's blood taken at the height of the fever. As Lebrede of Havana in his publications reported that he had observed viability up to 7 months of *Leptospira icteroides* in cultures made on the Noguchi medium, we continued to make inoculations of guinea pigs, and subsequent cultures with the heart's blood, at intervals of from 3 to 6 months, and are today—more than 3 years later—still in possession of living cultures of this particular strain.

Leptospira icterohæmorrhagiæ, which by our present methods cannot be distinguished morphologically from *Leptospira icteroides*, was likewise inoculated and cultured. No means of differentiation between the two types of leptospira was apparent at first. But later on, differences in respect to the duration of life of the two types of cultures became evident, and these differences increased as modifications in the culture media were undertaken.

The cultures of *Leptospira icterohæmorrhagiæ* during the early months were grown on Ungermann medium; later on, we employed for one half of the cultures a medium made of inactivated rabbit serum and physiological salt solution in the proportion of 1: 3; and finally the heart's blood only was used in physiological salt solution, a process which appears to have been first employed by Uhlenhuth and Zuelzer. In a number of instances, cultures were made with rabbit serum which had not been inactivated, but this method was abandoned, since as a rule

the results thus obtained were not as good as when the original Ungermann method was employed.

About 100 cultures of each of the two types—*Leptospira icteroides* and *Leptospira icterohæmorrhagiæ*—were made according to the original Ungermann technique; about an equal number of cultures with inactivated rabbit serum and physiological salt solution, 1:3; and 50 cultures each in physiological salt solution. In a few cases, a 1:3 mixture of uninactivated rabbit serum and physiological salt solution was used. The employment of the last mentioned medium did not give as consistent results as the mixture made with inactivated rabbit serum, although one of these *Leptospira icteroides* cultures proved viable after 2 years and 5 months.

It is noteworthy that when a small quantity of rabbit blood was added to the mixture of rabbit serum and physiological salt solution, the growth of *Leptospira icterohæmorrhagiæ* was abundant, while on the other hand the cultures of *Leptospira icteroides* in this medium did not as a rule show a great increase of growth. But neither of the two types of spirochete was viable for particularly long periods of time when grown in this medium.

Tables I and II show the growth periods of the two types in various media, the longest periods being recorded. The terms *Leptospira icteroides* and *icterohæmorrhagiæ* have been employed because they are current in Central and South America.

The conclusions to be drawn from these tables are:

1. In a medium made of physiological salt solution, in which *Leptospira icteroides* is known to grow less vigorously than *Leptospira icterohæmorrhagiæ*, the longest period of growth for *Leptospira icteroides* was $6\frac{1}{2}$ months, i.e. a period about one-half to two-thirds that of *Leptospira icterohæmorrhagiæ*, which was alive after 11 months.

2. In the Ungermann culture medium, *Leptospira icteroides* lived nearly a year longer (3 years in all) than *Leptospira icterohæmorrhagiæ* (2 years and 1 month).

3. With the use of inactivated rabbit serum and physiological salt solution, 1:3, *Leptospira icteroides* survived six times as long (3 years and $6\frac{2}{3}$ months) as *Leptospira icterohæmorrhagiæ* ($6\frac{2}{3}$ months), although the growth of the latter was abundant.

In judging these cultivation experiments, account must be taken of the fact that a comparison has been made in each case with but a single strain of the two types of leptospira. In order to arrive at

TABLE I.

Leptospira icteroides.

No.	Guinea pig	Dates cultures were made	Medium	Date last examined	No. of surviving cultures	Viability	
						yrs.	mos.
1	2	9/24/24	Isotonic salt solution	11/ 6/24	1		1½
2	3	9/24/24	" " "	11/ 6/24	1		1½
3	4	5/26/24	" " "	9/ 8/24	1		3½
4	5	12/20/24	" " "	5/31/25	1		6½
5	6	5/14/25	Blood; inactivated rabbit serum, isotonic salt solution	3/ 5/26	1		9½
6	7	1/25/24	Inactivated rabbit serum, isotonic salt solution	12/23/24	1		11
7	8	3/12/26	Ungermann	5/27/27	1	1	2
8	2	9/24/24	Inactivated rabbit serum, isotonic salt solution	3/11/26	1	1	6
9	9	9/11/23	Ungermann	5/19/25	1	1	8
10	10	3/10/24	Inactivated rabbit serum, isotonic salt solution	3/ 5/26	1	1	11
11	9	9/11/23	Ungermann	10/ 5/25	2	2	
12	11	12/ 1/23	"	3/ 5/26	1	2	3
13	12	12/22/24	Active rabbit serum, isotonic salt solution	5/27/27	1	2	5
14	13	12/ 1/23	Ungermann	12/10/26	1	3	
15	7	1/25/24	Inactivated rabbit serum, isotonic salt solution	5/27/27	1	3	4
16	14	11/ 5/23	Inactivated rabbit serum, isotonic salt solution	5/27/27	1	3	6½

TABLE II.

Leptospira icterohæmorrhagæ.

No.	Guinea pig	Dates cultures were made	Medium	Date last examined	No. of surviving cultures	Viability	
						yrs.	mos.
1	15	12/20/24	Isotonic salt solution	5/19/25	2		6
2	16	5/26/24	Inactivated rabbit serum, isotonic salt solution	12/17/24	1		6½
3	17	8/22/24	Isotonic salt solution	5/19/25	1		9
4	18	5/26/24	" " "	4/29/25	2		11
5	19	11/30/23	Ungermann	5/19/25	1	1	5½
6	20	11/30/23	"	5/19/25	1	1	5½
7	21	3/10/24	"	3/ 6/26	1	2	
8	22	1/22/24	"	2/26/26	1	2	1

really conclusive results, it will be necessary to make comparative studies of many strains of both types. The above mentioned results, however, may serve to indicate a direction for further investigation, since they suggest a possible means of differentiation of the organisms.

As the course of the infection with *Leptospira icteroides* in the experimental guinea pig has been minutely described by Noguchi, we shall not take it up here. The clinical history and the pathological anatomy of the animals have moreover been extensively dealt with by W. H. Hoffmann of Havana. On the basis of careful experimental work, the last mentioned author maintained for a long time a skeptical attitude regarding the etiological significance of *Leptospira icteroides* in yellow fever, because of the clinical and pathological similarities to *icterohæmorrhagiæ* infections in guinea pigs. Finally, however, even Hoffmann, on the basis of his studies of human yellow fever cases, of the Pfeiffer reaction, and of the specificity of the yellow fever vaccine and serum, appears no longer to doubt that *Leptospira icteroides* of Noguchi is the active, causative agent of yellow fever.

With regard to *Leptospira icteroides* from the guinea pig, the following points which Noguchi has already elucidated appear to be significant:

As a rule, the temperature attains its greatest height (40–41°C. and over) from the 4th to the 7th days after inoculation, while the peak is only occasionally attained on the 3rd day, or on the 8th to 11th days. Sometimes, however, the temperature may go as high as 40°C. on even the 14th day after inoculation. Temperatures up to 41°C. and over, are, however, not necessarily indicative of a severe infection. Guinea Pigs 23, 24, and 11 inoculated with *Leptospira icteroides* recovered completely after very high temperature rises. The first two animals showed the typical icterus. On the other hand, guinea pigs may succumb in a few days with a temperature of less than 40°C., the temperature suddenly falling below normal. In such cases, icterus, typical cutaneous hemorrhages, epistaxis, necrosis of the liver, and lime casts in the kidneys were all noted.

It is desirable to take the material for cultivation—heart's blood, suspension of liver or kidney—at the height of the fever, if possible when the temperature has reached 40° or over, and to place it immediately in the culture medium. According to our experience, this high body temperature, which is most favorable for insuring a vigorous culture growth, continues for only 1 or 2 hours and does not progress after the initial rise has been attained. Particularly is this the case in infections produced with avirulent cultures. Hence it is necessary in such instances to watch the animals closely in order to utilize a favorable opportunity for obtaining blood or other material.

The earliest period at which we were able to obtain positive results from cultures was 5 days after planting. As a rule, however, an abundant growth could not be obtained before about the 10th day. It happened not infrequently that culture media which appeared to be sterile for a number of weeks began to show signs of growth after about a month.

The temperature most favorable to the growth of cultures ranged between 24° and 34°C., but cultures kept at temperatures below 30°C. retained their viability for a longer time, for several weeks at least. If kept between 22° and 24°C., the organisms remain viable for a long period.

CONCLUSIONS.

The most noteworthy point observed in our studies is the extraordinary duration of life and the relatively meagre requirements for sustenance of *Leptospira icteroides*. It is conceivable that under natural conditions opportunities might arise for the prolonged existence of *Leptospira icteroides*, so that possibly after a lapse of years, the disease might reappear without introduction from outside. At any rate, the great viability of *Leptospira icteroides* must be considered in this connection.

A decrease in the virulence of the leptospira does not rule out the possibility of a renewed outburst of yellow fever, since Uhlenhuth and Zuelzer have shown that it is possible to set up Weil's disease in guinea pigs by means of apparently saprophytic spirochetes obtained from drinking water, when the virulence of these organisms has been artificially heightened.

In conclusion the writer takes pleasure in expressing his appreciation for the collaboration in this work of Dr. Bernardo Samper Sordo.